

FIG. 1-1

Constitutively Active Receptors

File Name	Receptor	Mutation Site	Sequence	Assay / Cells	Reference
CLASS A GROUP I					
MSHR_mouse	melanocyte-stimulating hormone MSH	TMII	92 VSVLETTIIL SEQ ID NO: 2 K	adenylyl cyclase activity/ HEK293, stably transfected	(Robbins, Nadeau et al. 1993)
CLASS A GROUP II SH1B_human	5-hydroxytryptamine <sub>1B</sub>	C-terminus of IC3	313 RERKATKTLGI SEQ ID NO: 3 K, R, Q	binding of [ <sup>35</sup> S]GTP[S] / CHO-K1	(Pauwels, Gouble et al. 1999)
SH2A_human	5-hydroxytryptamine <sub>2A</sub>	C-terminus of IC3	322 NEQKAGKVLGI SEQ ID NO: 4 K	IP production / COS-7	(Egan, Herrick-Davis et al. 1998)
2H2C_rat	5-hydroxytryptamine <sub>2C</sub>	C-terminus of IC3	312 NEDDAGKVLGI SEQ ID NO: 5 L	PI hydrolysis / COS-7	(Herrick-Davis, Egan et al. 1997)



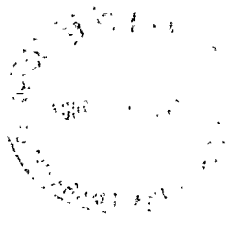
CLASS A GROUP II						
A1AB_human	$\alpha_{1B}$ -adrenergic alpha 1B-AR	TMDI  junction between TMDIII and IC2	63 FAIVGNILVIL SEQ ID NO: 6 A  142 CAISIDRYIGV SEQ ID NO: 7 A	IP / COS-7	(Scheer, Fanelli et al. 1997)	
A1AB_human	$\alpha_{1B}$ -adrenergic	junction between TMDIII and IC2	143 CAISIDRYIGV SEQ ID NO: 8 K	IP / COS-7	(Scheer, Costa et al. 2000)	
A1AB_human	alpha 1B-AR $\alpha_{1B}$ -adrenergic	TMDIII	128 AVDVLGCTASI SEQ ID NO: 9 F	IP / COS-1	(Perez, Hwa et al. 1996)	
		carboxyl end of IC3	293 REKKA <del>A</del> KTLLGI SEQ ID NO: 10 E	IP arachidonic acid release	(Hwa, Gaivin et al. 1997)	
		TMV	204 EPPFYALFSSLG SEQ ID NO: 11 V	IP / COS-1		
A1AB_human	$\alpha_{1B}$ -adrenergic	C-terminal IC3	293 SREKKA <del>A</del> KT SEQ ID NO: 12 X=19 different substitutions	PI / COS-7	(Kjelsberg, Cotecchia et al. 1992)	
A1AB_human	$\alpha_{1B}$ -adrenergic	C-terminus IC3	288 293 KFSREKKA <del>A</del> KTLLGI SEQ ID NO: 13 K H L	PI hydrolysis / rat fibroblast	(Allen, Lefkowitz et al. 1991)	
A2AA_human	$\alpha_2C10$ -adrenergic	C-terminal IC3 loop	373 (348?) EKRF <del>T</del> TVLAV SEQ ID NO: 14 X=F, A, C, E, K	adenylyl cyclase inhibition / HEK293	(Ren, Kurose et al. 1993)	
ACM1_human	alpha-2AAR muscarinic Hm1	C-terminal IC3 loop junction	360 SLVKEKKA <del>A</del> RTLS SEQ ID NO: 15 A	PI / HEK(U293)	(Högger, Shockey et al. 1995)	
ACM2-human	muscarinic acetylcholine M1 muscarinic acetylcholine M2	junction of IC3 and TMV1	390 KKVTRTL <del>1</del> A SEQ ID NO: 16 1-4 A inserted	IP production, inhibition of cAMP production / COS-7	(Liu, Blin et al. 1996)	

FIG. 1-3

CLASS A GROUP II						
ACM3_rat	m3 muscarinic (rat)	TMVI		507 TWTPYNIMVLVNT SEQ ID NO: 17 S	IP / COS-7	(Blüml, Mutschler et al. 1994)
ACM5_human	muscarinic acetylcholine M3 m5 muscarinic	N-terminus to TMII		chimera composed of m2 1-69 m5 77-445 m2 391-466	$\beta$ -gal / NIH 3T3	(Burststein, Spalding et al. 1996)
ACM5_human	muscarinic acetylcholine M5	TMVI		451 459 465 AIIILA FIIITW TPYNI MVLVST M L H C V S F T	$\beta$ -gal; radioligand binding / NIH-3T3	(Spalding, Burststein et al. 1998)
ACM5_human	m5 muscarinic muscarinic acetylcholine M5	TMVI	SEQ ID NO: 18	465 YNIIMVLVSTFCDCVCV SEQ ID NO: 19 X=V,F,R,K,+more	$\beta$ -gal; radioligand binding / NIH-3T3	(Spalding, Burststein et al. 1997)
ACM5_human	m5 muscarinic muscarinic acetylcholine M5	junction of TMVI and EC3		389 RKAFOGILLCCA SEQ ID NO: 20 R	adenylyl cyclase; agonist binding / CHW	(Mason, Moore et al. 1999)
B1AR_human	$\beta_1$ -adrenergic	C-terminus		266 272 FCLKEHKALKTLGI SEQ ID NO: 21 SR K A	adenylyl cyclase activation; agonist binding affinity / COS-7 or CHO	(Samama, Cotecchia et al. 1993); (Lefkowitz, Cotecchia et al. 1993)
DADR_human	dopamine D1A	carboxyl terminal IC3		264 SFKMSEKRETKVLKT SEQ ID NO: 22 I K 288 from D1B receptor APDTSIKKETKVLKT SEQ ID NO: 23	adenylyl cyclase; cAMP accumulation / HEK293	(Charpentier, Jarvie et al. 1996)
DADR_human	dopamine D1	TMVI		286 FVCCWLPFFIL SEQ ID NO: 24 A	cAMP accumulation / COS-7	(Cho, Taylor et al. 1996)
HH2R_rat	histamine H <sub>2</sub>	IC2		115 FMISLDRYCAV SEQ ID NO: 25 N, A	cAMP production / HEK-293	(Alewijne, Timmerman et al. 2000)

FIG. 1-4

File Name	Receptor	Mutation Site	Sequence	Assay / Cells	Reference
CLASS A GROUP III					
OPSD_human	opsin	TMII	90 FMVLGGFTSTLY SEQ ID NO: 26 D	transducin; phosphorylation by rhodopsin kinase / COS	(Rim and Oprian 1995)
	rhodopsin	TMIII	113 GCNLEGGFFAT SEQ ID NO: 27 Q		
		TMVII	292 296 MTIPAFFAKSAAY SEQ ID NO: 28 E G, E, M 293 Ala neutral a.a converted to carboxylate and competes with <sup>113</sup> Glu for salt bridge with <sup>296</sup> Lys		
OPSD_human	opsin	TMIII	134 VVLAIERYVVW SEQ ID NO: 29 I, Q, S	transducin; radioligand binding / COS	(Acharya and Karnik 1996)
OPSD_human	rhodopsin	TM6	257 RMVLIIMVIAFL SEQ ID NO: 30 Y, N	transducin, GTPγS uptake / COS	(Han, Smith et al. 1998)
OPSD_human	opsin	plus TM3 TMVII	plus G113Q 296 PAFFAKSAAY SEQ ID NO: 31 G X=E,M natural mutants + 10 different a.a. substitutions disrupts critical salt bridge between <sup>296</sup> Lys(TMVII) and <sup>113</sup> Glu(TMIII)	transducin; radioligand binding / COS	(Govardhan and Oprian 1994); (Cohen, Yang et al. 1993)
	rhodopsin	IC2	134 VVLAIERYVVW SEQ ID NO: 32 Q		(Cohen, Yang et al. 1993)



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FIG. 1-5

TRFR_mouse	thyrotropin-releasing hormone TRH-R	carboxyl tail	335 FRKLQCKQK STOP	<sup>45</sup> Ca <sup>2+</sup> efflux, [Ca <sup>2+</sup> ] / Xenopus oocytes; IP formation / AIT20, <i>stably transfected</i>	(Matus-Leibovitch, Nussenzveig et al. 1995)
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FIG. 1-6

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File Name	Receptor	Mutation Site	Sequence	Assay / Cells	Reference
CLASS A GROUP IV					
BRB2_human	bradykinin B <sub>2</sub>	TMIII	113 AIIISMNLYSSI SEQ ID NO: 34	IP production / COS-7	(Marie, Koch et al. 1999)
	B2 bradykinin BK-2	TMVI	256 LLFLICWLPFQI SEQ ID NO: 35 F		

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FIG. 1-7

File Name	Receptor	Mutation Site	Sequence	Assay / Cells	Reference
CLASS A GROUP V					
AG2R_rat	AT <sub>1A</sub> Type-1A angiotensin II	TMIII	111 ASVSFNL <sup>111</sup> YASV SEQ ID NO: 36 A disrupts <sup>111</sup> Asn(TMIII) - <sup>111</sup> Tyr(TMVII) interaction	phospholipase C; IP production / COS-7	(Grobowski, Maigret et al. 1997)
AG2R_rat	AT <sub>1A</sub>	C-terminus of TM7	305 LFYGF <sup>305</sup> LGGKEK SEQ ID NO: 37 Q	IP production / HEK-293; intracellular Ca <sup>2+</sup> mobilization / CHO	(Pamot, Bardin et al. 2000)
FM1R_human	Type-1A angiotensin II formylmethionylleucylphenylalanine (fMLPR)	IC1 other multiple mutations SEQ ID NO: 38 SEQ ID NO: 39	51 LVIVVAGFEMTH <sup>51</sup> VTTISYLNKAVA LVVWVTAFAEKRTINAIWFLNLAVA (K above conflicts with SWISS-PROT database)	PI production; phospholipase C stimulation / COS-7	(Amatruda, Dragas-Graonic et al. 1995)
IL8B_human	interleukin-8 receptor B CXCR-2 chemokine	IC2	138 ACISVD <sup>138</sup> RYLAIVH SEQ ID NO: 40 V	IP production; Ca <sup>2+</sup> mobilization and actin polymerization / NIH 3T3	(Burger, Burger et al. 1999)
LSHR_human	luteinizing hormone (LH)	IC3	564 MATNK <sup>564</sup> DTIAKK SEQ ID NO: 41 G	cAMP production / HEK293	(Kudo, Osuga et al. 1996)
LSHR_human	luteinizing hormone (LH)	TMVI	578 ILIFT <sup>578</sup> DFTCWA SEQ ID NO: 42 G	cAMP production / COS-7	(Shenker, Laue et al. 1993)
LSHR_human	luteinizing hormone (LH)	TM6	571 577 KIAKK <sup>571</sup> MAILLIFT <sup>577</sup> DFTCM I I	cAMP production / COS-7	(Kosugi, Van Dop et al. 1995)
LSHR_rat	luteinizing hormone / human chorionic gonadotropin (LH/hCG)	TMVI	556 ILLIFT <sup>556</sup> DFTCWA SEQ ID NO: 44 G, Y	cAMP production / HEK 293T	(Bradbury, Kawate et al. 1997; Bradbury and Menon 1999)
OPRD_mouse	delta opioid receptor	TM3	128 KVLSD <sup>128</sup> YNNMF SEQ ID NO: 45 A, K, H	adenyl cyclase inhibition / COS-7	(Cavalli, Babey et al. 1999)
OXYR_human	oxytocin	IC2	137 LMSLD <sup>137</sup> RLAIC SEQ ID NO: 46 A	IP production / COS-7	(Fanelli, Barbier et al. 1999)

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FIG. 1-8

PAFR_human	platelet-activating factor (PAF)	C-terminus of IC3	231 EVKRRALMMVCTVLAV SEQ ID NO: 47	IP production / COS-7	(Parent, Le Gouill et al. 1996)
PAFR_human	platelet-activating factor (PAF)	TMIII	100 CLFFINTYCSV SEQ ID NO: 48 A	arachidonate release, IP production, adenylyl cyclase inhibition / CHO	(Ishii, Izumi et al. 1997)
PE23_human	prostaglandin E <sub>2</sub> , EP3III EP3IV	C-terminal tail	360 FCQEEFWGN SEQ ID NO: 49 FCQMRKRRLREQEEFWGN SEQ ID NO: 50 ↑truncated	inhibition of adenylyl cyclase / CHO-K1	(Jin, Mao et al. 1997)
PE23_mouse	prostaglandin E <sub>2</sub> EP3	carboxyl-terminal tail SEQ ID NO: 51	336 KILLRRKFCQIRDHT (3α) MMNHL (3β) ↑truncated	inhibition of adenylyl cyclase / CHO, stably expressed	(Hasegawa, Negishi et al. 1996)
THR_human	thrombin	EC2 loop SEQ ID NO: 52	259 268 CHDVLTNETLLEGYYAYY DLKD KDF I	<sup>45</sup> Ca <sup>2+</sup> efflux, PI hydrolysis, reporter gene induction / COS-7	(Nanevicz, Wang et al. 1996)
TSHR_human	thyrotropin (TSHR) thyroid stimulating hormone	EC1 EC2	486 YYNHAIDWQTG SEQ ID NO: 53 F, M 568 YAKVSI <sup>T</sup> CLPMD SEQ ID NO: 54	inositol phosphate-- diacylglycerol cascade / COS-7	(Parma, Van Sande et al. 1995)
TSHR_human	thyrotropin (TSHR) thyroid stimulating hormone	TMIII TMVII	509 ASELSVYTLTV SEQ ID NO: 55 A 672 YPLNSCAMPFL SEQ ID NO: 56 Y	adenylyl cyclase activation / COS-7	(Duprez, Parma et al. 1994)
TSHR_human	thyrotropin (TSHR)	TMV	597 VAFVIVCCCHV SEQ ID NO: 57 L	cAMP formation / COS-7 cells	(Esapa, Duprez et al. 1999)
TSHR_human	thyroid stimulating hormone thyrotropin (TSHR)	TMVII	677 CANPFLVAIFT SEQ ID NO: 58 V	cAMP formation / CHO cells	(Russo, Wong et al. 1999)
TSHR_human	thyroid stimulating hormone thyrotropin (TSHR) thyroid stimulating hormone	IC3	613 621 VRNPQYNPGDKDTIAK deletion SEQ ID NO: 59	cAMP formation / COS-7	(Wonerow, Schoneberg et al. 1998)



FIG. 1-9

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TSHR_human	thyrotropin (TSHR)	IC3 / TMVI	SEQ ID NO: 60	623 KDTKIAKRMVAVLIFTDFICM V I	632 I	cAMP activation / COS-7	(Paschke, Tonacchera et al. 1994)
V2R_human	thyroid stimulating hormone vasopressin V2	IC2	SEQ ID NO: 61	136 LAMTLDRHRAI A		cAMP formation / COS-7	(Morin, Cotte et al. 1998)

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FIG. 1-10

File Name	Receptor	Mutation Site	Sequence	Assay / Cells	Reference
CLASS B GROUP I					
CALR_human	human calcitonin hCTR-1 hCTR-2	wild type (native) protein		adenylyl cyclase cAMP production / COS-1	(Cohen, Thaw et al. 1997)
CLASS B GROUP II					
PTRR_human	parathyroid hormone PTH / PTH-related peptide	junction of IC1 and TMII  junction of IC3 and TMVI	223 TRNYIHMLFL SEQ ID NO: 62 R, K  410 KLKSTLVLMF SEQ ID NO: 63 C, others	cAMP accumulation / COS-7	(Schipani, Jensen et al. 1997)
CLASS B GROUP III					
GIPR_human	glucose-dependent insulinotropic peptide (GIP-R)	TMVI	340 VFAPVTEEQAR SEQ ID NO: 64 P	cAMP production / L293	(Tseng and Lin 1997)
GLR_rat	glucagon	junction of IC loop I and TMII  IC end of TMVI	178 TRNYIHGNLFA SEQ ID NO: 65 R  352 RLARSTLTLP SEQ ID NO: 66 A	cAMP accumulation / COS-7	(Hjorth, Orskov et al. 1998)
VIPR_human	vasoactive intestinal peptide 1 (VIP)	junction of IC loop 1 and TMII  junction of IC loop 3 and TMVI	178 RNYIHMLFI SEQ ID NO: 67 R functional integrity of the N-terminal EC domain  343 LARSTLTLP SEQ ID NO: 68 X= K, P	cAMP production / COS-7 or CHO	(Gaudin, Maoret et al. 1998) (Gaudin, Rouyer-Fessard et al. 1998)

FIG. 1-11

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File Name	Receptor	Mutation Site	Sequence	Assay / Cells	Reference
CLASS C					
CASR_human	calcium-sensing	N-terminal EC	<p> <u>TL</u> <u>S</u> <u>F</u> <u>V</u> <u>A</u> <u>Q</u> <u>N</u> <u>K</u> <u>I</u> <u>D</u> <u>S</u> <u>I</u> <u>N</u> <u>L</u> <u>D</u> <u>E</u> <u>F</u> <u>C</u> <u>N</u> <u>C</u> <u>S</u> <u>E</u> <u>H</u> </p> <p>various substitutions, in multiple combinations</p>	<p>IP / tsA</p> <p>SEQ ID NO: 69</p>	(Jensen, Spalding et al. 2000)

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FIG. 1-12

File Name	Receptor	Mutation Site	Sequence	Assay / Cells	Reference
CLASS D					
O74283 RCB2 <i>C. cinereus</i>	pheromone	TM6	229 PLSAYQIYLGT SEQ ID NO: 70 P	heterologous yeast assay	(Olesnicky, Brown et al. 1999)
STE2_yeast	pheromone $\alpha$ -factor	TM6	258 QSLVPSIIFI SEQ ID NO: 71 LL	<i>lacZ</i> reporter gene	(Konopka, Margarit et al. 1996)
STE2_yeast	pheromone $\alpha$ -factor	double mutations TM5 and TM6	223 MSFVLVVKILLAIR SEQ ID NO: 72 C C 247 251 DSFHILLVCQSLL SEQ ID NO: 73 CC CC double mutations added double mutations	<i>lacZ</i> reporter gene / yeast	(Dube, DeCostanzo et al. 2000)
STE3_yeast	pheromone $\alpha$ -factor	IC3	194 DVRDIHCTNS SEQ ID NO: 74 Q	$\beta$ -galactosidase	(Boone, Davis et al. 1993)
STE2_yeast	pheromone $\alpha$ -factor	TM6	253 258 LIMSCQSLVPSIIFI SEQ ID NO: 75 L LP	$\beta$ -galactosidase	(Sommers, Martin et al. 2000)



## FIG. 1-13

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## FIG. 1-14

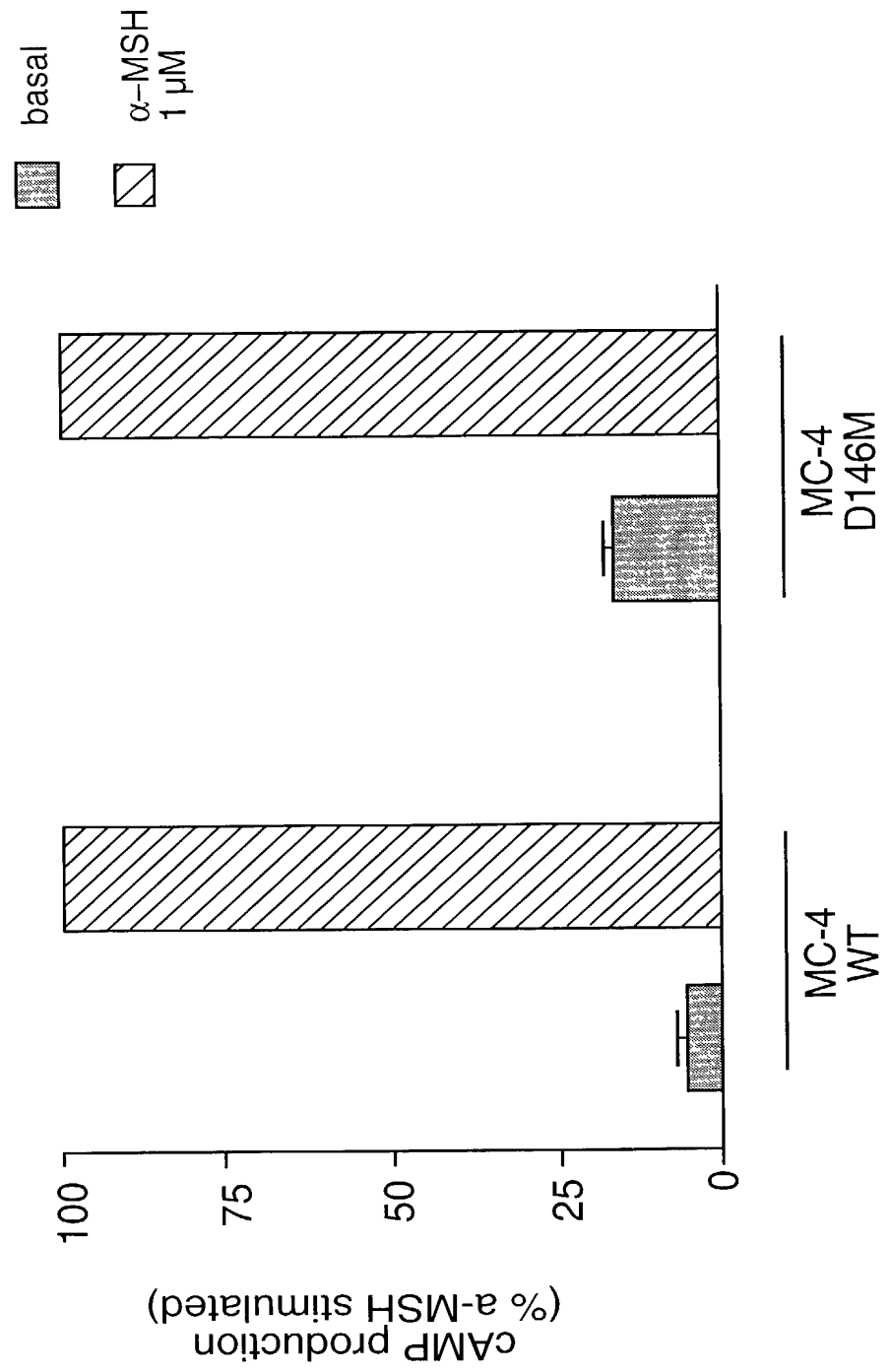
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## FIG. 1-15

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FIG. 2

A Point Mutation Enhances MC-4 Receptor  
Constitutive Activity



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10039545 1050302



FIG. 3

Light Emission Induced by the WT CCK-BR  
vs. a Constitutively Active Mutant

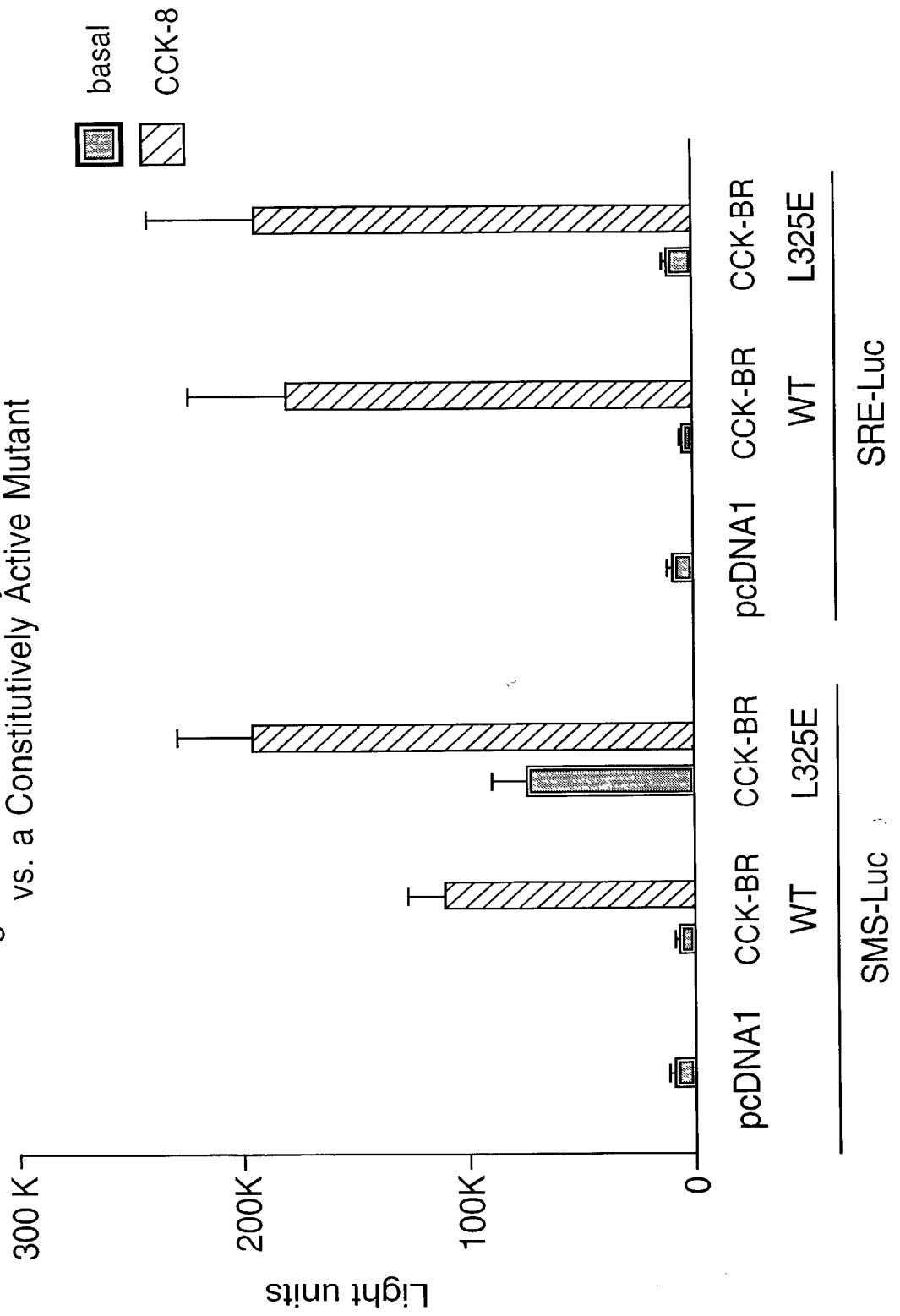


FIG. 4

A Point Mutation Confers Constitutive Activity to the Rat  $\mu$  Opioid Receptor

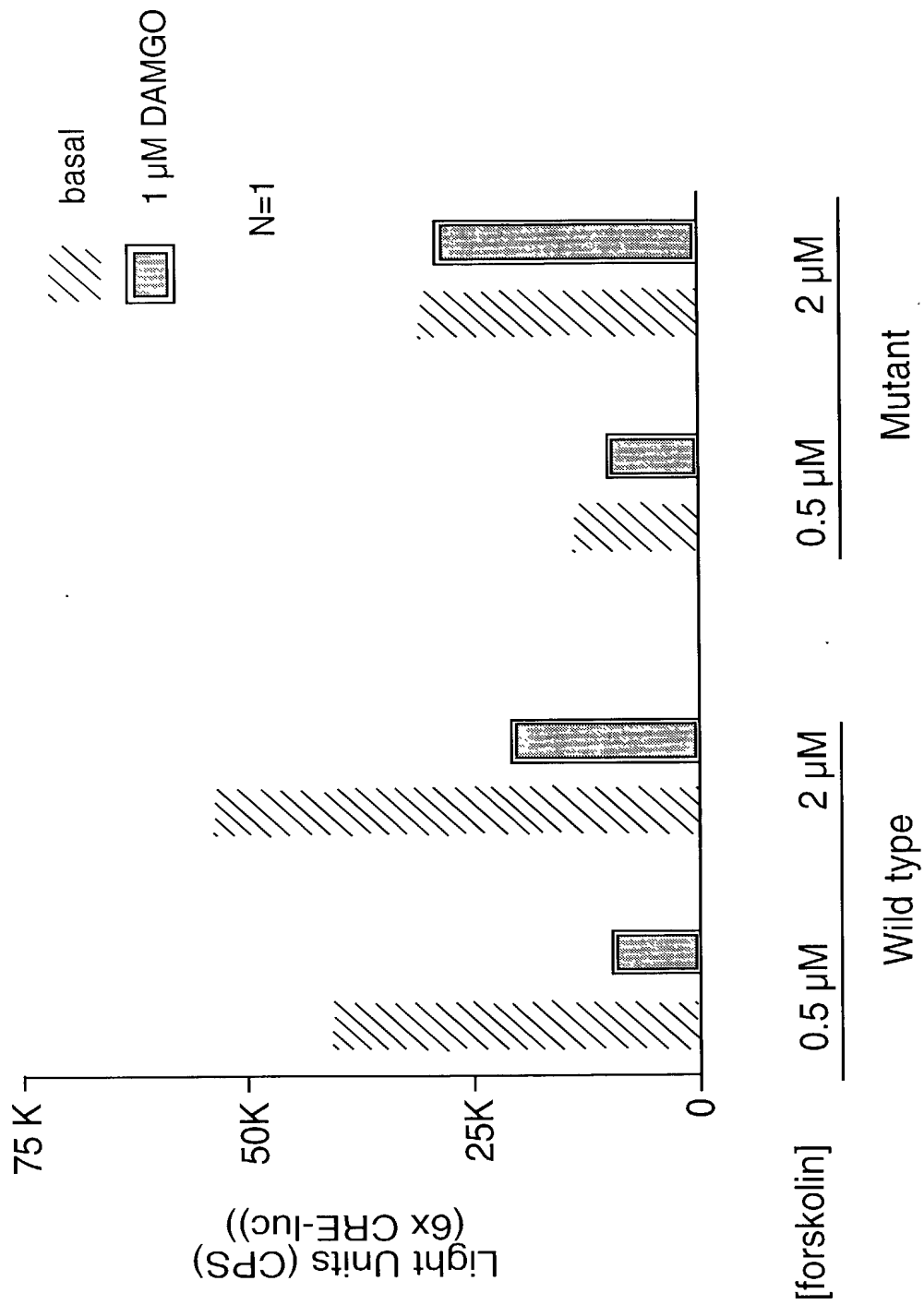


FIG. 5

Forskolin Stimulated HEK293 Cells Transfected  
With pcDNA1 and a CRE-luc Construct

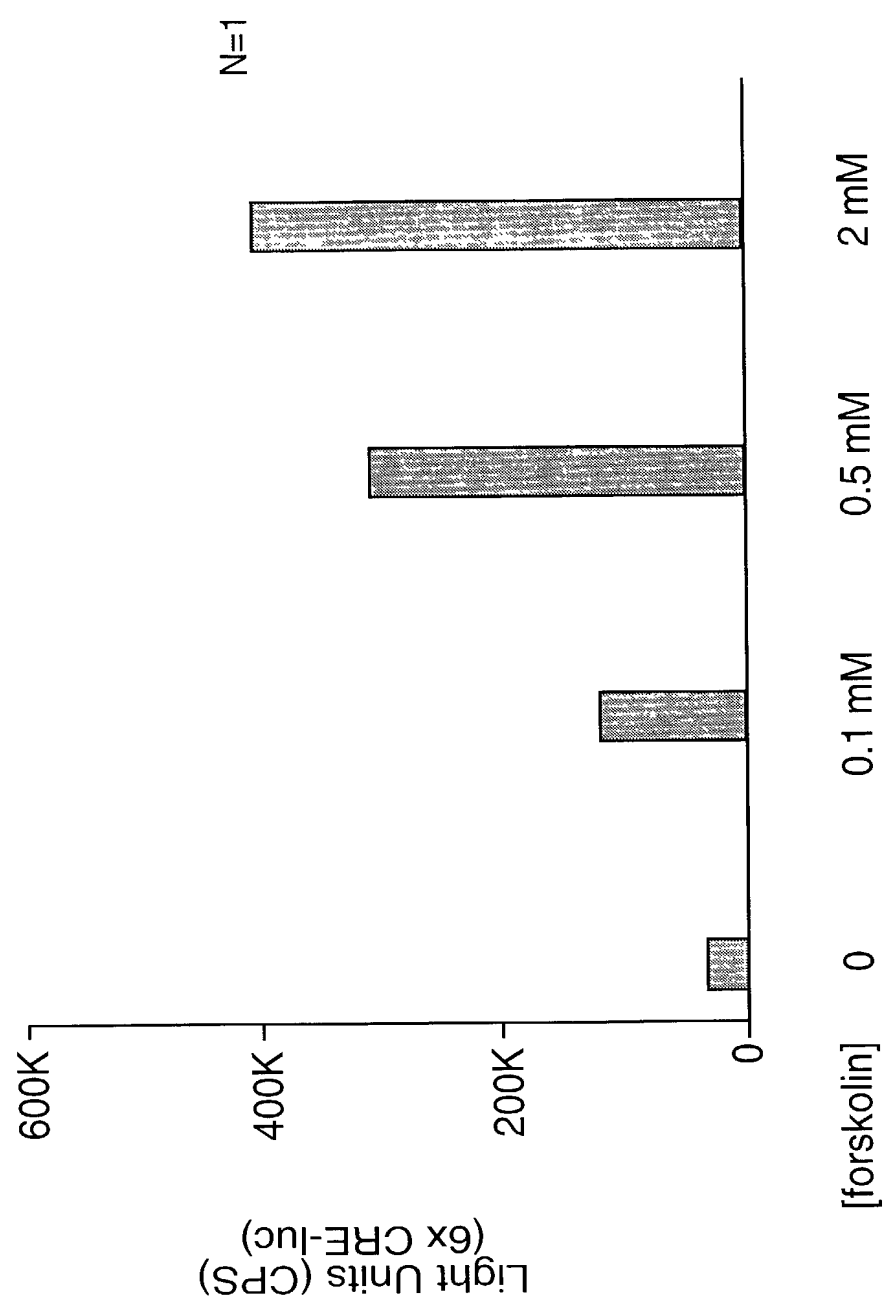
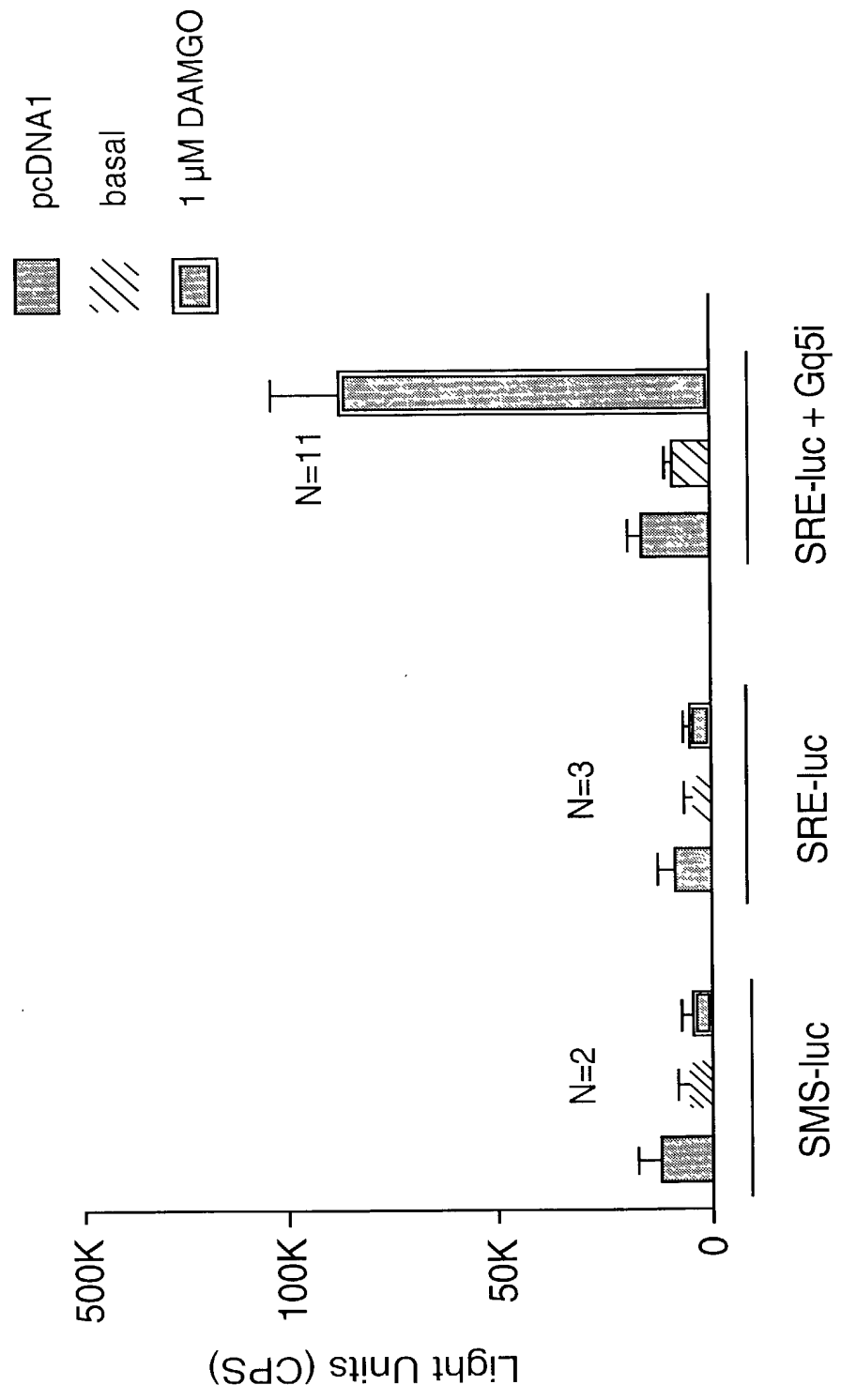


FIG. 6

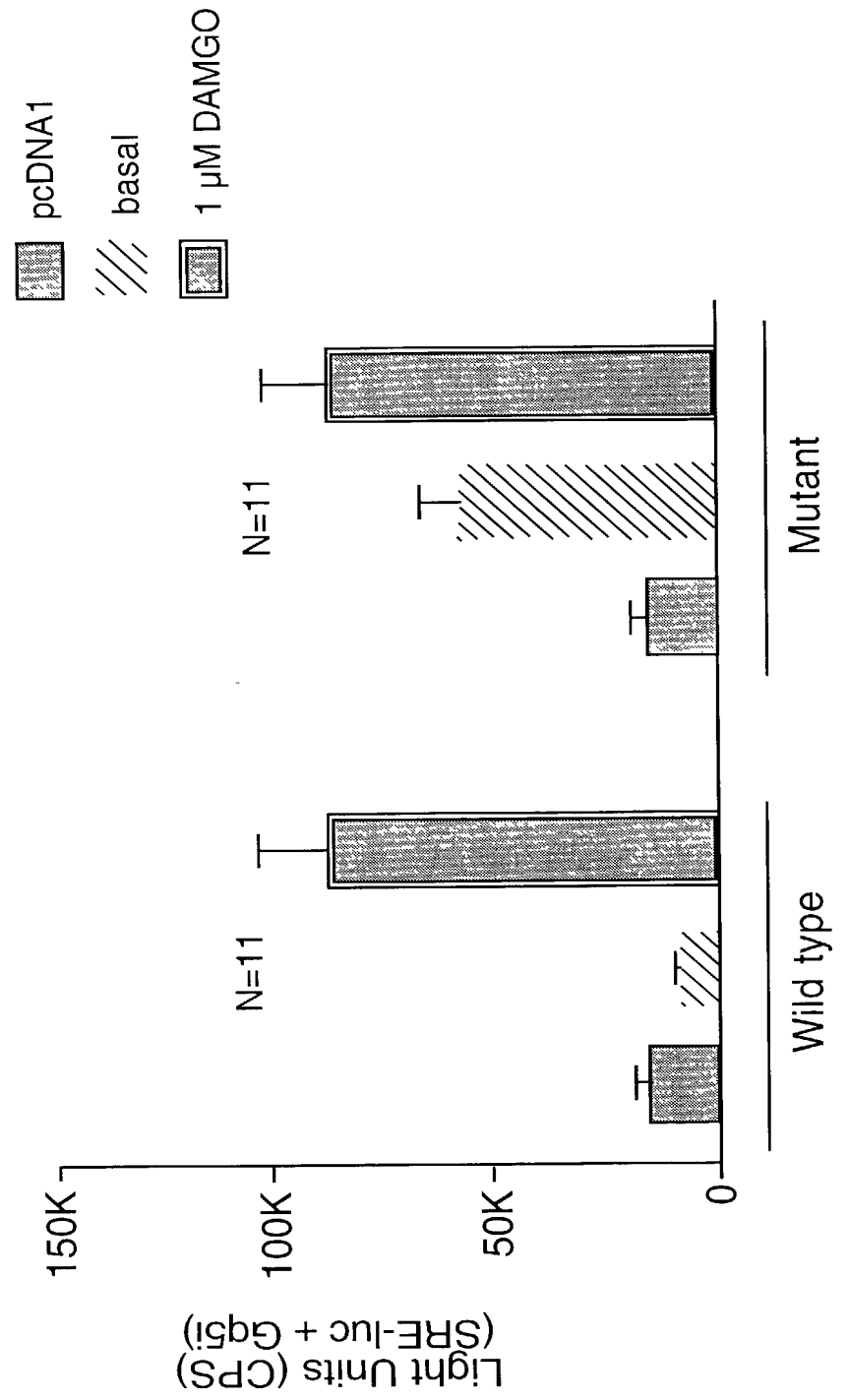
The Rat  $\mu$  Opioid Receptor Signals Through G $\alpha$ i



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FIG. 7

A Point Mutation Confers Constitutive Activity to the Rat  $\mu$  Opioid Receptor



F/G.  
8

## Target Residues Within Class I GPCRs

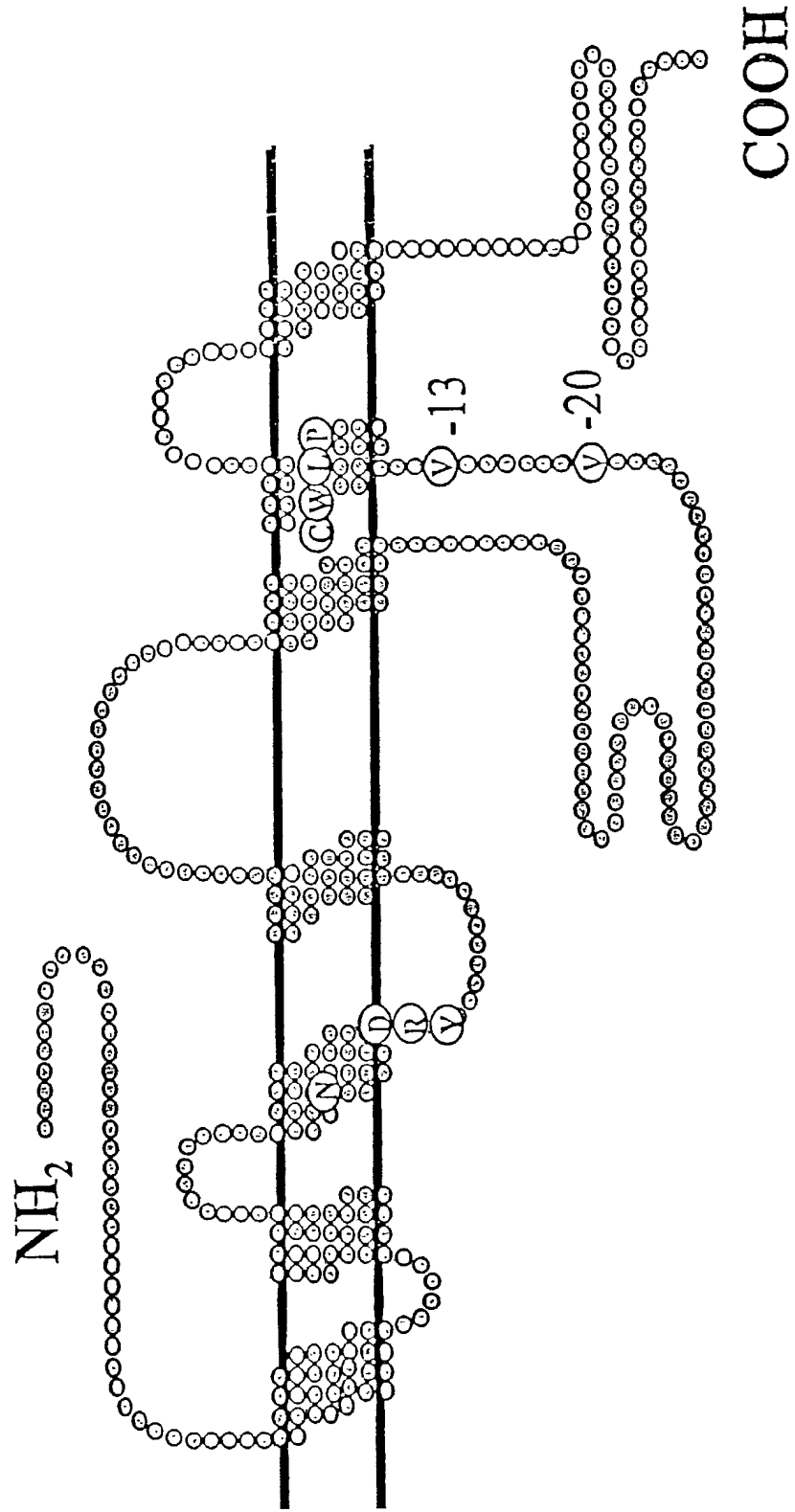
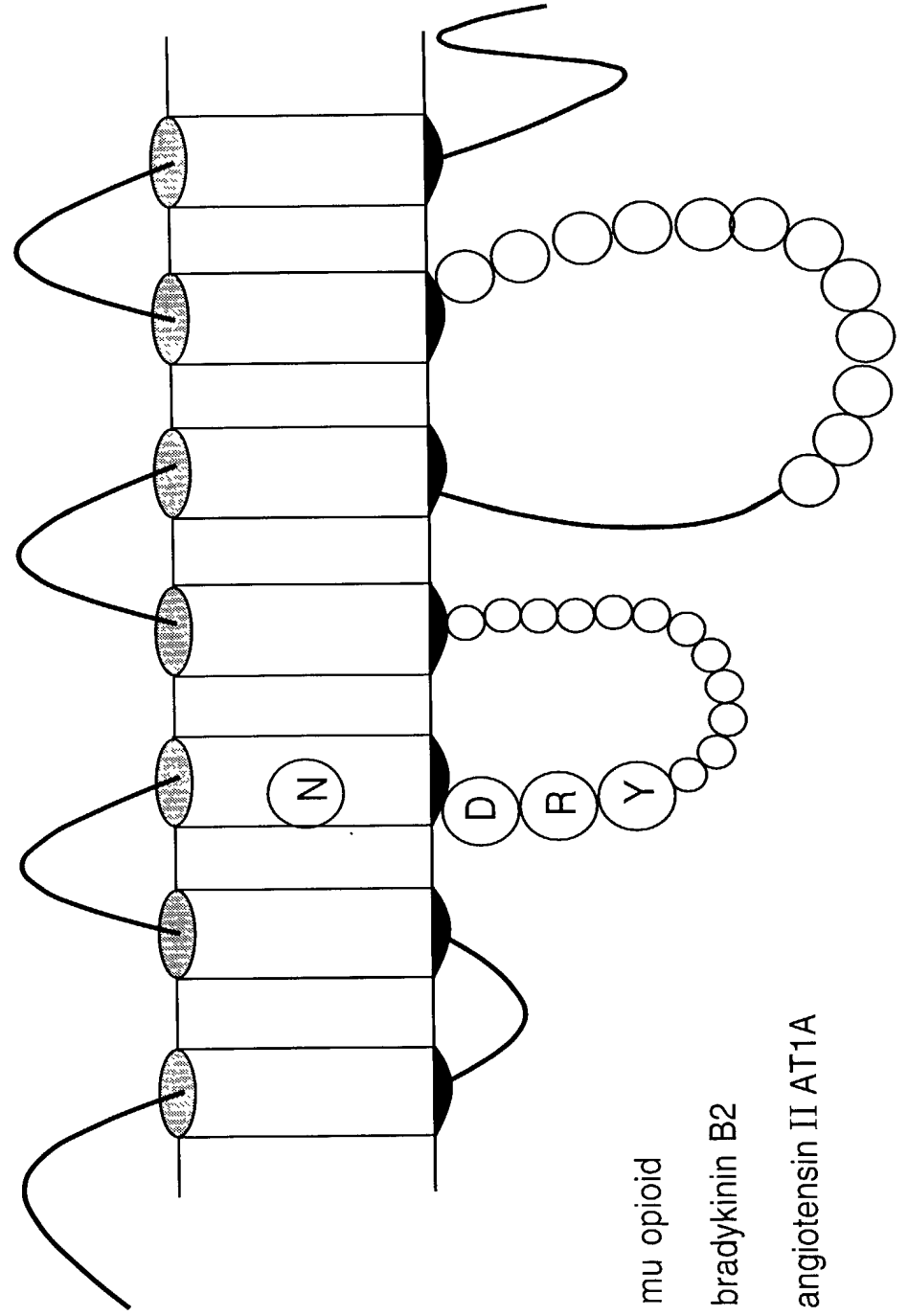


FIG. 9

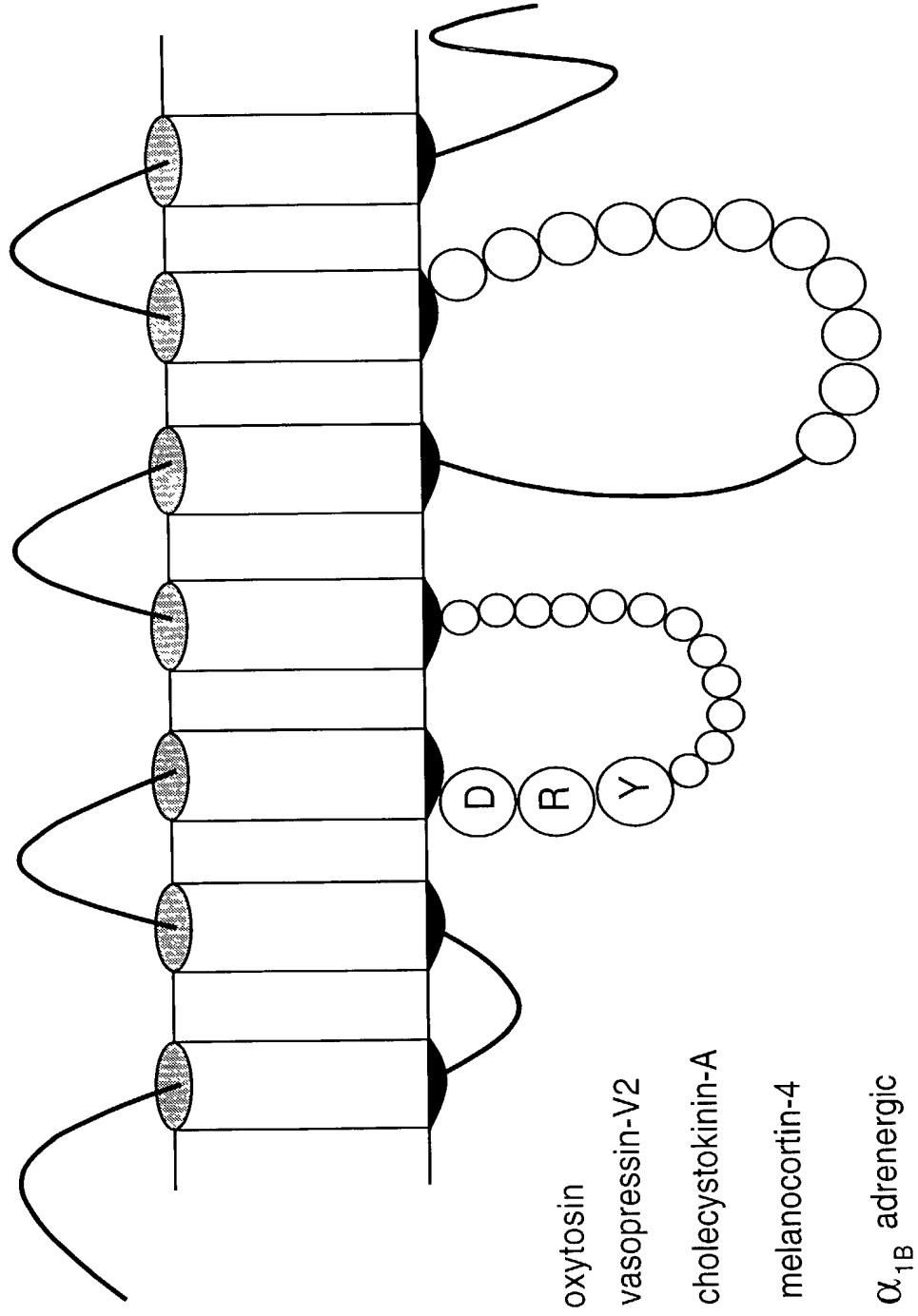
TMD III Asn (-14 from DRY) is a Target  
for Mutation Induced Constitutive Activity



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FIG. 10

The 'DRY' Motif is a Target for Mutation  
Induced Constitutive Activity





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FIG. 11

A Point Mutation Enhances MC-4 Receptor  
Constitutive Activity

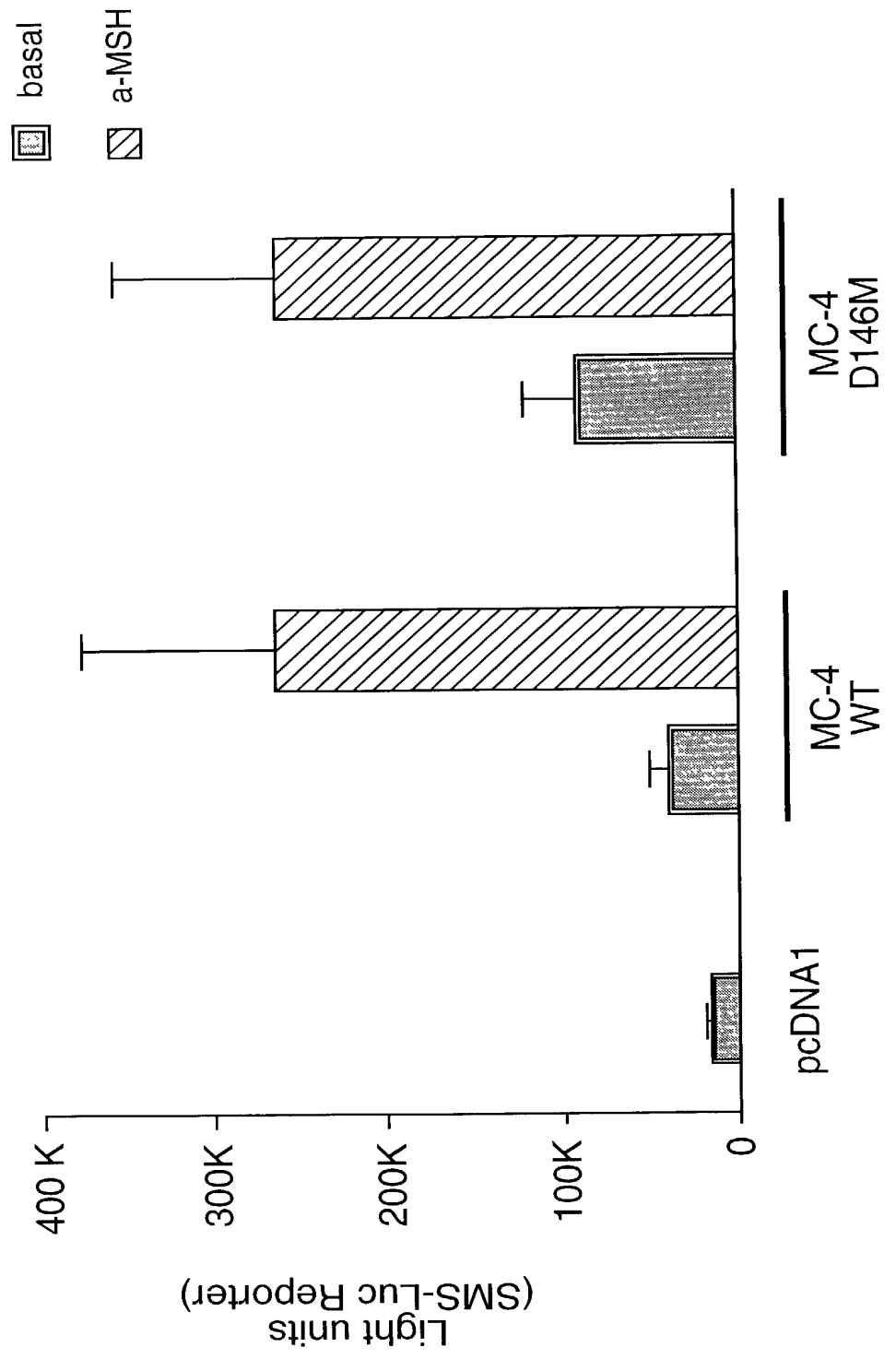
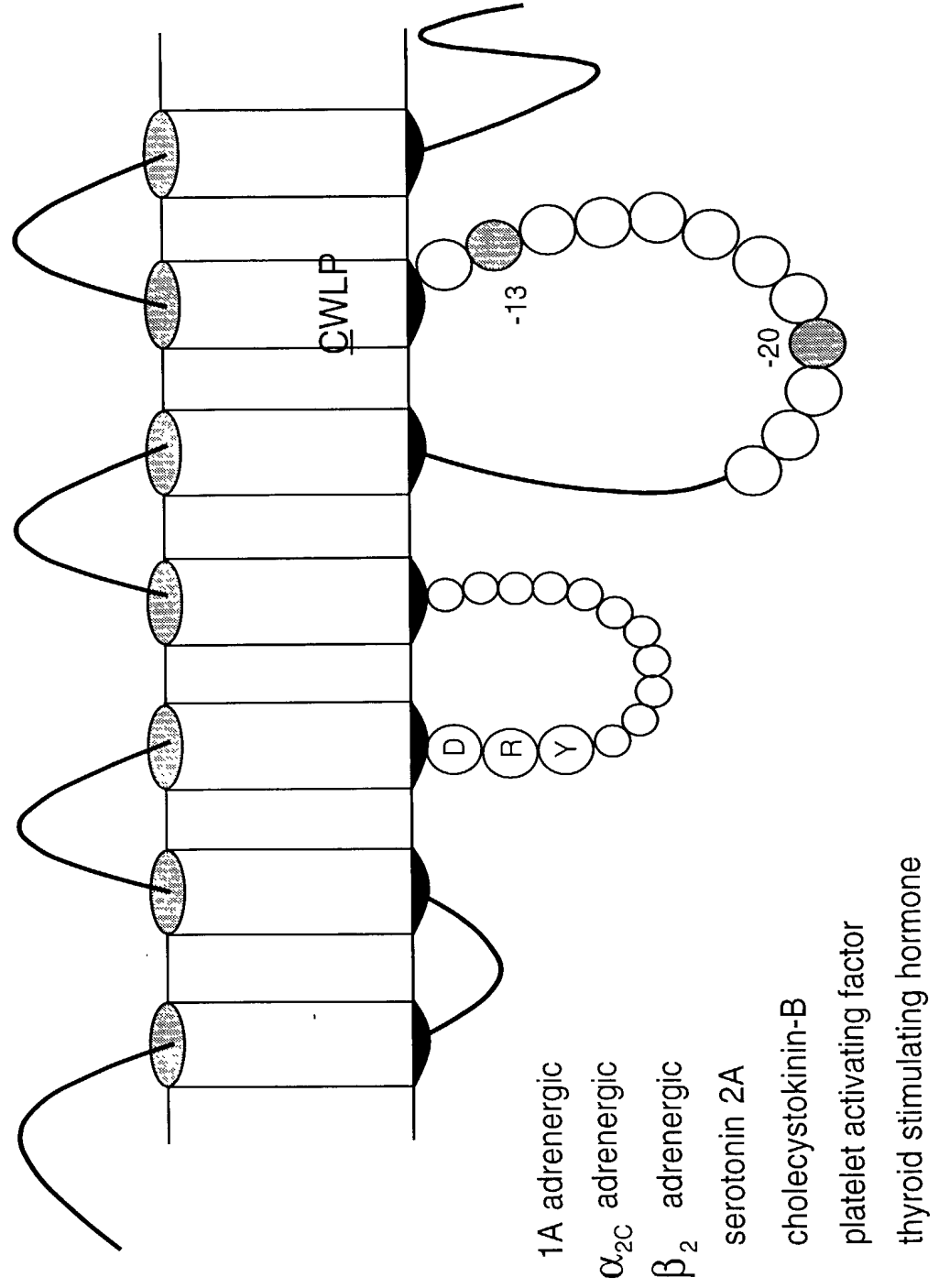


FIG. 12

The -13 Position is a Target for Mutation  
Induced Constitutive Activity



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FIG. 13

SEQ ID NO: 76 ork 1 -----MESPIQIFRGEPTCAPSACLPPNSSAWFPGWAEF..DSNGSAGSEDAQ  
 SEQ ID NO: 77 orkr 1 -----MESPIQIFRGEPTCAPSACLPPNSSWFPNWAES..DSNGSVGSEDCQ  
 SEQ ID NO: 78 orm 1 MDSSAAPTNASNCTDAAYSSCSFAPSPGSWY..NLSHLDGNLSDPCGPNRTDLGGRLSL  
 SEQ ID NO: 79 ormr 1 MDSSTGPGNTSDCSDPFAQASCSFA..PGSWL..NLSHVDGNQSDPCGLNRTGLGGNDSL  
 SEQ ID NO: 80 ord 1 -----MEAPSAGABL..Q..PPLFANASDAYPSACPSAGANASG  
 SEQ ID NO: 81 AT1a 1 -----MALNSSAEDGIKRIC  
 SEQ ID NO: 82 BK-2 1 -----MFSPWKISMFLSVREDSVPTTASFSADMLNVTLOQPTLNS.TFAQ

ork 49 LEPAEISPAH..PVHITANYSVFVVLGNGSLVMEVILTRYTKMKTATNIYIFNLALADA  
 orkr 49 LEPAEISPAH..PVHITANYSVFVVLGNGSLVMEVILTRYTKMKTATNIYIFNLALADA  
 orm 59 CPPTGS.PSMITAITIMALYSIVCVGLFGNPLVMYVIVRYTKMKTATNIYIFNLALADA  
 ormr 57 CPQTGS.PSMVTAITIMALYSIVCVGLFGNPLVMYVIVRYTKMKTATNIYIFNLALADA  
 ord 37 PPGASASSIALATAITIMALYSIVCAVGLFGNPLVMYVIVRYTKMKTATNIYIFNLALADA  
 AT1a 16 DDCPAGRHSYIFVMIPTLYSIFVGLFGNPLVIVIVFYMKKTIVASVFTLNALADL  
 BK-2 45 SKCPQVEWLGWNLTIQPPFLWVFEVLALENIFVLSVFLCHKSSCTVAEIVYGNLAAADL

ork 107 LVTHITMPFQSTVYLMN..EWPFQDVLCKIVISIDYNNMFTSIFTLTMSVDRYIAVCHPVK  
 orkr 107 LVTHITMPFQSAVYLMN..EWPFQDVLCKIVISIDYNNMFTSIFTLTMSVDRYIAVCHPVK  
 orm 118 LATSITLFPQSNVYLMG..IWPFGTILCKIVISIDYNNMFTSIFTLTMSVDRYIAVCHPVK  
 ormr 116 LATSITLFPQSNVYLMG..IWPFGTILCKIVISIDYNNMFTSIFTLTMSVDRYIAVCHPVK  
 ord 97 LATSITLFPQSAKYLMG..IWPFGTILCKIVISIDYNNMFTSIFTLTMSVDRYIAVCHPVK  
 AT1a 76 CFLLTLELWAVYTAMEYRWPFGNHLCKIASAVTENLYASVELLTCHSTDRYIAIVHPMK  
 BK-2 105 ILACGLEFWAITISNPNFNLFEGETLCRVNNAISMNLYSSICFLMLVSDRYIAIVKTM

-14 from DRY      \*

ork 166 ALDFRTELKAKIINICIWHLSSVGHSAIVLGGTKVR..EDVDVIECSLOFFEDDYSWWD  
 orkr 166 ALDFRTELKAKIINICIWHLSSVGHSAIVLGGTKVR..EDVDVIECSLOFFEDDYSWWD  
 orm 177 ALDFRTERNAKINONWILSSAIGLPVFMFTTKYR..C..GSIDCLTFSHPTW.YWE  
 ormr 175 ALDFRTERNAKINONWILSSAIGLPVFMFTTKYR..C..GSIDCLTFSHPTW.YWE  
 ord 156 ALDFRTEAKAKIINICIWHLSSVGHSAIVLGGTKVR..D..GAVVCMLOFFSPSW.YWD  
 AT1a 136 SRLRRIMLVAKVTCIIITWLAGLASPAVTHRN..YFIENNTNIVCAFHYESRN.STLP  
 BK-2 165 MGRMRGVRWAKYYSIVWGCALLLSSPMLVFRMTKEYSDEGHNVTAIVISYPS...LIWE

ork 224 IFMKICVFIFAFVLPVLIITVCYTLMLRLKSVRLSGSREKDRNLRRITRMVLVVAVF  
 orkr 224 IFMKICVFIFAFVLPVLIITVCYTLMLRLKSVRLSGSREKDRNLRRITRMVLVVAVF  
 orm 232 NLTKICVFIFAFVLPVLIITVCYGLMLRLKSVRLSGSREKDRNLRRITRMVLVVAVF  
 ormr 230 NLTKICVFIFAFVLPVLIITVCYGLMLRLKSVRLSGSREKDRNLRRITRMVLVVAVF  
 ord 211 TVTKICVFIFAFVLPVLIITVCYGLMLRLKSVRLSGSREKDRNLRRITRMVLVVAVF  
 AT1a 193 IGLGLTKNLGLFLEPFLIITSYTLTKALKKAYELQKNKPRND...IFRTIMAVLFEF  
 BK-2 222 VFTNMLENVVGFLLE..LSVITFCTMCHMOVLRRNNEOKFKEIQTE..RRATVLVLVLLF

ork 284 IVCWTPPIHIFILVEALGS.T....SHSTAALSSYFICIALGYTNSSLNPLVYAFLDENF  
 orkr 284 IVCWTPPIHIFILVEALGS.T....SHSTAALSSYFICIALGYTNSSLNPLVYAFLDENF  
 orm 292 IVCWTPPIHIVIIKALVTIP.....ETTFQTVSWHFCIALGYTNSSLNPLVYAFLDENF  
 ormr 290 IVCWTPPIHIVIIKALVTIP.....ETTFQTVSWHFCIALGYTNSSLNPLVYAFLDENF  
 ord 271 IVCWAPIHIFVIVWTLVDID....RRDPLVVAALHLICIALGYANSSLNPLVYAFLDENF  
 AT1a 250 FFSWVPHQIFTFLEVLQGLVIHDCIKISDIVDTAMPITICTAYFNNCLNPLFYGLGKKF  
 BK-2 280 ITCWLPFQISTFLDTLHRLGILSSQDERIIDVITQIASFMAYSNSCLNPLVYVITGKRF

ork 338 KRCFRIFCFPLKMRMEROSTSRVF.NTVOD..PAYLRDIDGMNKPV-----  
 orkr 338 KRCFRIFCFPIKMRMEROSTSRVF.NTVOD..PASMRDVGGMNKPV-----  
 orm 346 KRCFRIFCFPTSSNHEQONSTRVRONT..RDHPSTANTVDRTNHOLENLEAETAPLP  
 ormr 344 KRCFRIFCFPTSSNHEQONSTRVRONT..RDHPSTANTVDRTNHOLENLEAETAPLP  
 ord 326 KRCFRQLCRKPCGPPDPSSFSRAREATAREVVTACTPSDGGPGGAAAA-----  
 AT1a 310 KRYELQLLKYIIPPAKSHS...SLSTKM..STLSYRPSDNMSSSAKKPASCFEVE-  
 BK-2 340 RKKSWEVYQGVCOGGCRSEPIQMENS..GTL..RTSISVROTHKLQDWAGSRQ

## FIG. 14

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SEQ ID NO: 83 mORmouse 1 MDSSAGEGNISDCSDPLA.PASCSPA..PGSWLNLSHVDGNOSDPCGPNRTGLGGSLSLC  
 SEQ ID NO: 79 mORrat 1 MDSSSTGPGNTSDCSDPLA.QASCSPA..PGSWLNLSHVDGNOSDPCGPNRTGLGGSLSLC  
 SEQ ID NO: 84 mORbovin 1 MDSSGAVPTNASNCIDFTHPSSCSPAPSPSSWVNFHLEGNLSDPCGPNRTGLGGSLSLC  
 SEQ ID NO: 85 mORhuman 1 MDSSAAPTNASNCIDALAY.SSCSPAPEGSGWVNLHLDGNLSDPCGPNRTGLGGSLSLC  
 SEQ ID NO: 86 mORpig 1 MDSSADERNASNCIDPFSPSSMCSPVPSPSSWVNFHLEGNLSDPCGPNRTGLGGSLSLC  
 SEQ ID NO: 87 mORws 1 METS...GNISDFLYPLS.....NPMVS.....NSSVLCRNFSNSTSFLNMNGSSRSDTD  
 SEQ ID NO: 81 Atla 1 -----MALNSSAEDGKRIQDDG  
 SEQ ID NO: 82 BK-2 1 -----MFSFWKISMFLSVREDSVPTTASFADMLNVTLCPTLNG.TFAQSKC

mORmouse 58 PQTGSPSMYTAITIMALYSIVCVVGLFGNFLVMYVIVRYTKMKTATNIYIFNLALADALA  
 mORrat 58 PQTGSPSMYTAITIMALYSIVCVVGLFGNFLVMYVIVRYTKMKTATNIYIFNLALADALA  
 mORbovin 61 PSAGSPSMYTAITIMALYSIVCVVGLFGNFLVMYVIVRYTKMKTATNIYIFNLALADALA  
 mORhuman 60 PQTGSPSMYTAITIMALYSIVCVVGLFGNFLVMYVIVRYTKMKTATNIYIFNLALADALA  
 mORpig 61 PQTGSPSMYTAITIMALYSIVCVVGLFGNFLVMYVIVRYTKMKTATNIYIFNLALADALA  
 mORws 48 EODKTPVITAIITITLYSIVCVVGLVGNVVMYVIVRYTKMKTATNIYIFNLALADALA  
 Atla 19 EKACRHSYIFVM.IPTLYSIHFVVGLEGMSLVVIVYFYMKIKTVASVFNLALADALCF  
 BK-2 48 PQVEWLGWNTII.QPPFLWLVFLATLENIIVLSVFLCHKSSCTVAEIVLGNLAADLIL

mORmouse 118 TSTLPFQSVNYLMG.TWPFQNLCKIVISIDYNNMFTSIFTLCTMSVDRIYAVCHPVKAL  
 mORrat 118 TSTLPFQSVNYLMG.TWPFQNLCKIVISIDYNNMFTSIFTLCTMSVDRIYAVCHPVKAL  
 mORbovin 121 TSTLPFQSVNYLMG.TWPFQNLCKIVISIDYNNMFTSIFTLCTMSVDRIYAVCHPVKAL  
 mORhuman 120 TSTLPFQSVNYLMG.TWPFQNLCKIVISIDYNNMFTSIFTLCTMSVDRIYAVCHPVKAL  
 mORpig 121 TSTLPFQSVNYLMG.TWPFQNLCKIVISIDYNNMFTSIFTLCTMSVDRIYAVCHPVKAL  
 mORws 107 TSTLPFQSVNYLMG.TWPFQNVCKIVISIDYNNMFTSIFTLCTMSVDRIYAVCHPVKAL  
 Atla 78 LLTLPLWAVYTAMEYRWPFQNLCKIVASVTEHNTASVELTCLSDRIYAVCHPVKSR  
 BK-2 107 ACGLPEWATITISNNFDLFCETLCVYNHISMNLYSSICFIMLSDRIYAVCHPVKALSMG

mORmouse 177 DFRTPRNAKIVNCNWILSSAIGLPVMFMATTKYR.....GSIDCTLTFSSHPTWYWE  
 mORrat 177 DFRTPRNAKIVNCNWILSSAIGLPVMFMATTKYR.....GSIDCTLTFSSHPTWYWE  
 mORbovin 180 DFRTPRNAKIVNCNWILSSAIGLPVMFMATTKYR.....GSIDCTLTFSSHPTWYWE  
 mORhuman 179 DFRTPRNAKIVNCNWILSSAIGLPVMFMATTKYR.....GSIDCTLTFSSHPTWYWE  
 mORpig 180 DFRTPRNAKIVNCNWILSSAIGLPVMFMATTKYR.....GSIDCTLTFSSHPTWYWE  
 mORws 166 DFRTPRNAKIVNCNWILSSAIGLPVMFMATTKYR.....GSIDCTLTFSSHPTWYWE  
 Atla 138 LRRTMLVAKVTCTIIMMAGLASLPVIRNV....YFIENTNITVCAFHYESRSTLP  
 BK-2 167 RMRGVFWAKYSLVINGCTLLSSPMLVFRIMK...EYSDEGHNVTAQVISYPS..LIWE

mORmouse 230 NLLKICVFIFAFIMPVLIITVCYGLMILRLKSVRMLSGSKEKDRNLRRITRMVLVVAVF  
 mORrat 230 NLLKICVFIFAFIMPVLIITVCYGLMILRLKSVRMLSGSKEKDRNLRRITRMVLVVAVF  
 mORbovin 233 NLLKICVFIFAFIMPVLIITVCYGLMILRLKSVRMLSGSKEKDRNLRRITRMVLVVAVF  
 mORhuman 232 NLLKICVFIFAFIMPVLIITVCYGLMILRLKSVRMLSGSKEKDRNLRRITRMVLVVAVF  
 mORpig 233 NLLKICVFIFAFIMPVLIITVCYGLMILRLKSVRMLSGSKEKDRNLRRITRMVLVVAVF  
 mORws 226 TLLKICVFIFAFIMPVLIITVCYGLMILRLKSVRMLSGSKEKDRNLRRITRMVLVVAVF  
 Atla 193 IGLGTQNLILGLFPLIILTSMTLWALKKAYEOKNKPEND...IFRIIMATVLLF  
 BK-2 222 VFTNMLNVVSELLP.LSVITFCTYQIMOLRNEQKFKETQTE.RRATMLVLVLLF

mORmouse 290 IVCWTPPIHIYVIAKALITI.....PETTFQTVSWHFCIALGYTNSCLNPVLYAFLDENF  
 mORrat 290 IVCWTPPIHIYVIAKALITI.....PETTFQTVSWHFCIALGYTNSCLNPVLYAFLDENF  
 mORbovin 293 IVCWTPPIHIYVIAKALITI.....PETTFQTVSWHFCIALGYTNSCLNPVLYAFLDENF  
 mORhuman 292 IVCWTPPIHIYVIAKALITI.....PETTFQTVSWHFCIALGYTNSCLNPVLYAFLDENF  
 mORpig 293 IVCWTPPIHIYVIAKALITI.....PETTFQTVSWHFCIALGYTNSCLNPVLYAFLDENF  
 mORws 286 IVCWTPPIHIYVIAKALITI.....PNSLFQTVSWHFCIALGYTNSCLNPVLYAFLDENF  
 Atla 250 FFSWVPHQISTFLDVLIOLGVHDKISDIVDTAMPITICTAYENNCNPLVYGLGKKK  
 BK-2 280 IVCWLPFQISTFLDTLHRLGILSSCOERIIDVITQIASPMAYSNSCLNPVLYVIGKRF

mORmouse 344 KRCFREFC..IPTSSSTIEQONSARIRONTREHPSTANTVDRTNHOLENLEAETAPLE  
 mORrat 344 KRCFREFC..IPTSSSTIEQONSTRIRONTREHPSTANTVDRTNHOLENLEAETAPLE  
 mORbovin 347 KRCFREFC..IPTSSSTIEQONSTRIRONTREHPSTANTVDRTNHOLENLEAETAPLE  
 mORhuman 346 KRCFREFC..IPTSSSTIEQONSTRIRONTREHPSTANTVDRTNHOLENLEAETAPLE  
 mORpig 347 KRCFREFC..IPTSSSTIEQONSARIRONTREHPSTANTVDRTNHOLENLEAETAPLE  
 mORws 340 KRCFREFC..UPSPSVLDLONSTRNSNPQCGQSSGHKVDNRNRV-----  
 Atla 310 KYFLCLLKYLIPKAKSHS...SLSTKMSLSYRPSDNVSSSAKKPASCFEVE----  
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FIG. 15

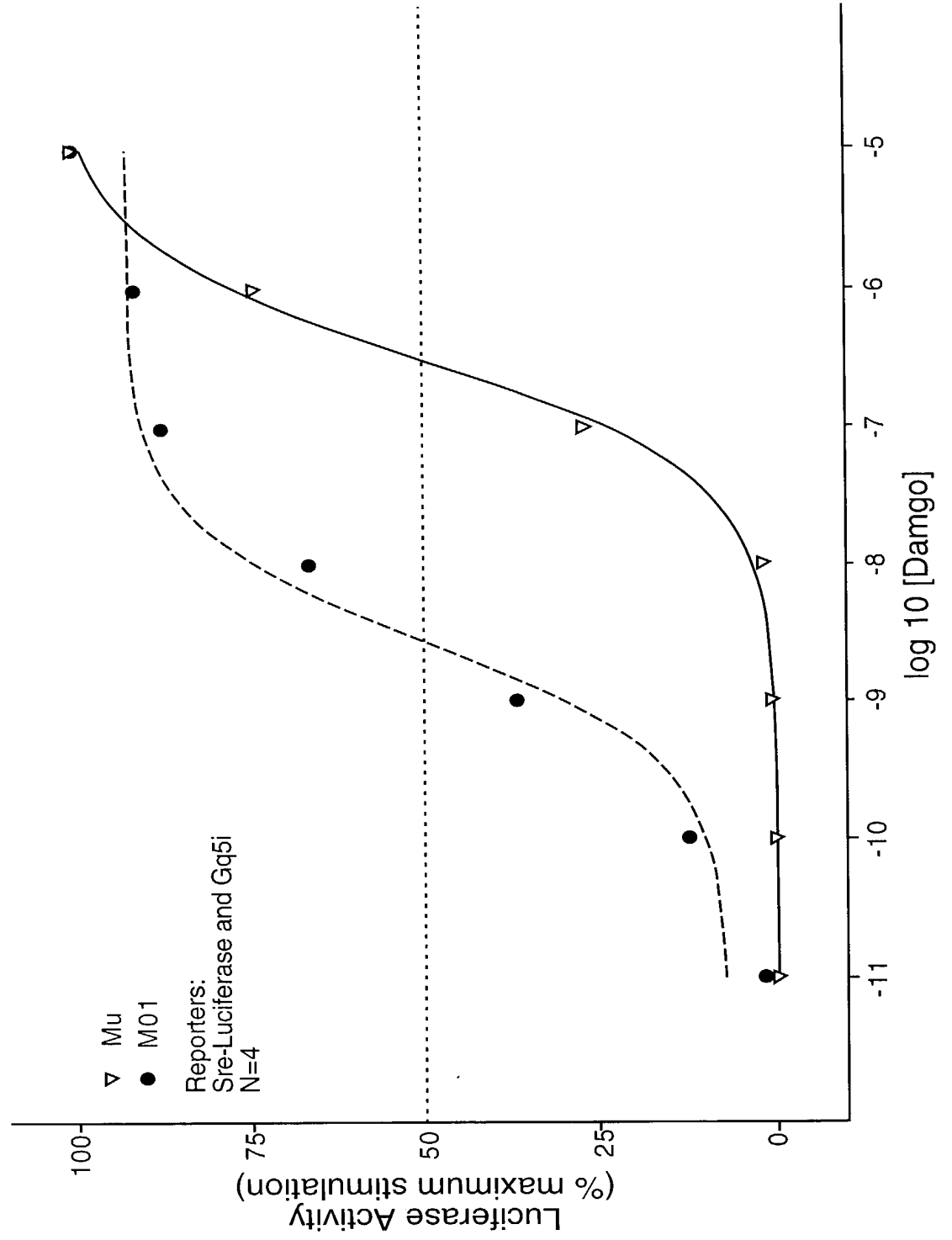
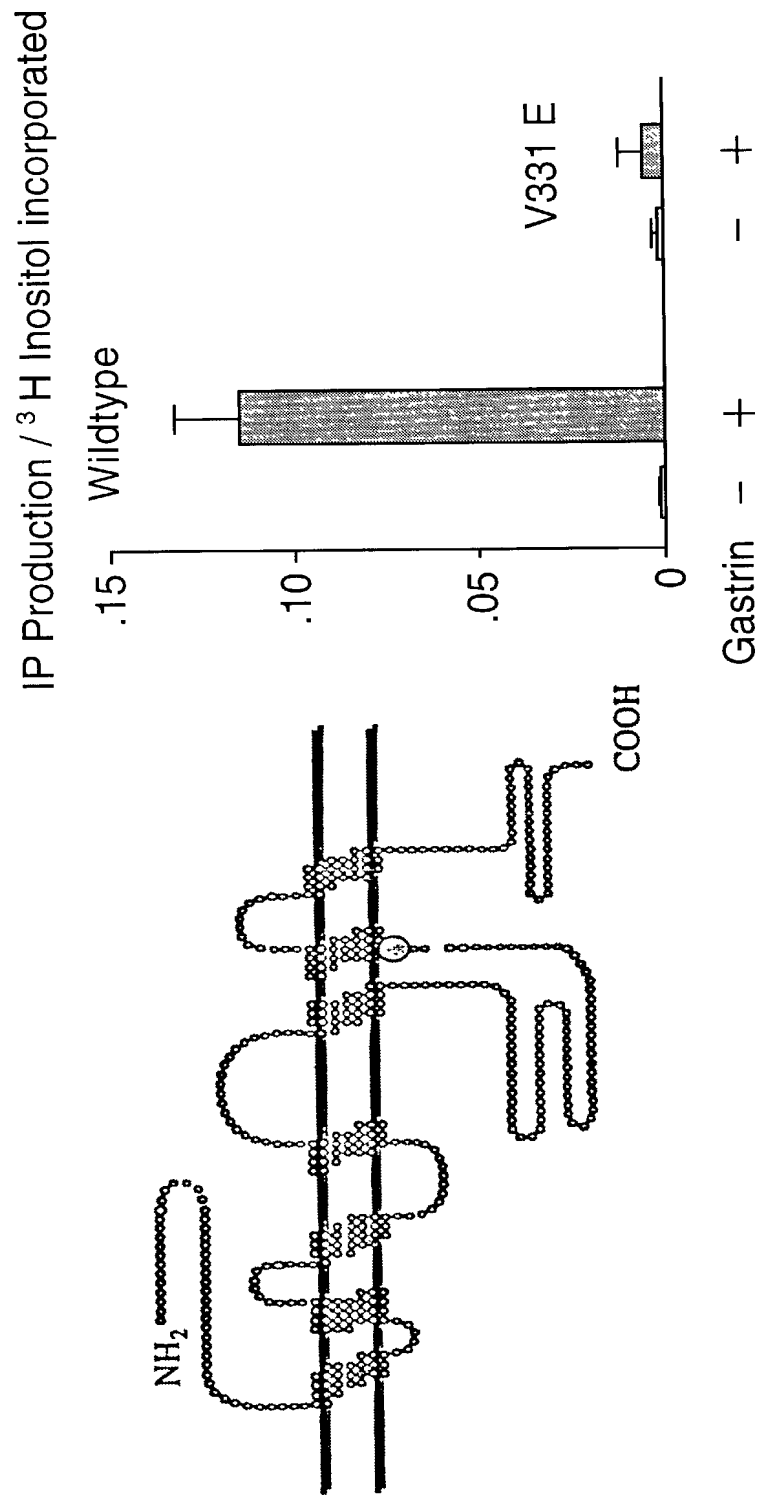


FIG. 16

An Intracellular Point Mutation Results in  
Loss of Ligand-Induced Function



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FIG. 17

